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COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION
0.21	0.21

FULL ESTIMATED COST

FILE 'MEDLINE' ENTERED AT 11:05:31 ON 07 AUG 2006

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FILE 'EMBASE' ENTERED AT 11:05:31 ON 07 AUG 2006

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=> s StcE protein

L1 11 STCE PROTEIN

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 9 DUP REM L1 (2 DUPLICATES REMOVED)

=> s l2 and (viscosity or cleaving or cleavage)

L3 5 L2 AND (VISCOSITY OR CLEAVING OR CLEAVAGE)

=> d l3 1-5 ibib ab

L3 ANSWER 1 OF 5 MEDLINE on STN

ACCESSION NUMBER: 2006372498 MEDLINE

DOCUMENT NUMBER: PubMed ID: 16788173

TITLE: Characterization of the StcE protease activity of  
Escherichia coli O157:H7.

AUTHOR: Grys Thomas E; Walters Laura L; Welch Rodney A

CORPORATE SOURCE: Department of Medical Microbiology & Immunology, University  
of Wisconsin-Madison, Room 481 MSC, 1300 University Ave.,  
Madison, WI 53706, USA.

CONTRACT NUMBER: 5T32GM08349 (NIGMS)  
R01 AI051735 (NIAID)

SOURCE: Journal of bacteriology, (2006 Jul) Vol. 188, No. 13, pp.  
4646-53.

Journal code: 2985120R. ISSN: 0021-9193.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200608

ENTRY DATE: Entered STN: 22 Jun 2006

Last Updated on STN: 5 Aug 2006

Entered Medline: 4 Aug 2006

AB The StcE zinc metalloprotease is secreted by enterohemorrhagic Escherichia coli (EHEC) O157:H7 and contributes to intimate adherence of this bacterium to host cells, a process essential for mammalian colonization. StcE has also been shown to localize the inflammatory regulator C1 esterase inhibitor (C1-INH) to cell membranes. We tried to more fully characterize StcE activity to better understand its role in EHEC pathogenesis. StcE was active at pH 6.1 to 9.0, in the presence of NaCl concentrations ranging from 0 to 600 mM, and at 4 degrees C to 55 degrees C. Interestingly, antisera against StcE or C1-INH did not eliminate StcE cleavage of C1-INH. Treatment of StcE with the proteases trypsin,

chymotrypsin, human neutrophil elastase, and *Pseudomonas aeruginosa* elastase did not eliminate StcE activity against C1-INH. After StcE was kept at 23 degrees C for 65 days, it exhibited full proteolytic activity, and it retained 30% of its original activity after incubation for 8 days at 37 degrees C. Together, these results show the StcE protease is a stable enzyme that is probably active in the environment of the colon. Additionally,  $k(\text{cat})/K(\text{m})$  data showed that StcE proteolytic activity was 2.5-fold more efficient with the secreted mucin MUC7 than with the complement regulator C1-INH. This evidence supports a model which includes two roles for StcE during infection, in which StcE acts first as a mucinase and then as an anti-inflammatory agent by localizing C1-INH to cell membranes.

L3 ANSWER 2 OF 5 MEDLINE on STN  
 ACCESSION NUMBER: 2005100592 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15731026  
 TITLE: The StcE protease contributes to intimate adherence of enterohemorrhagic *Escherichia coli* O157:H7 to host cells.  
 AUTHOR: Grys Thomas E; Siegel Matthew B; Lathem Wyndham W; Welch Rodney A  
 CORPORATE SOURCE: Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, 1300 University Ave., Room 481 MSC, Madison, WI 53706, USA.  
 CONTRACT NUMBER: 5T32GM08349 (NIGMS)  
 R01 AI051735 (NIAID)  
 SOURCE: Infection and immunity, (2005 Mar) Vol. 73, No. 3, pp. 1295-303.  
 Journal code: 0246127. ISSN: 0019-9567.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-AY074613; GENBANK-AY714880  
 ENTRY MONTH: 200504  
 ENTRY DATE: Entered STN: 1 Mar 2005  
 Last Updated on STN: 2 Apr 2005  
 Entered Medline: 1 Apr 2005

AB Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is a diarrheal pathogen that causes attaching and effacing (A/E) lesions on intestinal epithelial cells. Strains of the O157 serogroup carry the large virulence plasmid pO157, which encodes the etp type II secretion system that secretes the genetically linked zinc metalloprotease StcE. The Ler regulator controls expression of many genes involved in A/E lesion formation, as well as StcE, suggesting StcE may be important at a similar time during colonization. Our laboratory has previously demonstrated that StcE cleaves C1-esterase inhibitor, a regulator of multiple inflammation pathways. Here we report two new substrates for StcE, mucin 7 and glycoprotein 340, and that purified StcE reduces the viscosity of human saliva. We tested the hypothesis that StcE contributes to intimate adherence of EHEC to host cells by cleavage of glycoproteins from the cell surface. The fluorescent actin stain (FAS) test was used to observe the intimate adherence represented by fluorescently stained bacteria colocalized with regions of bundled actin formed on HEP-2 cells. An *E. coli* O157:H7 strain with a *stcE* gene deletion was not affected in its ability to generally adhere to HEP-2 cells, but it did score threefold lower on the FAS test than wild-type or complemented strains. Addition of exogenous recombinant StcE increased intimate adherence of the mutant to wild-type levels. Thus, StcE may help block host clearance of *E. coli* O157:H7 by destruction of some classes of glycoproteins, and it contributes to intimate adherence of *E. coli* O157:H7 to the HEP-2 cell surface.

L3 ANSWER 3 OF 5 MEDLINE on STN  
 ACCESSION NUMBER: 2002378601 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12123444

TITLE: StcE, a metalloprotease secreted by Escherichia coli  
0157:H7, specifically cleaves C1 esterase inhibitor.  
AUTHOR: Lathem Wyndham W; Grys Thomas E; Witowski Sarah E; Torres  
Alfredo G; Kaper James B; Tarr Phillip I; Welch Rodney A  
CORPORATE SOURCE: Department of Medical Microbiology and Immunology,  
University of Wisconsin, Madison, WI 53706, USA.  
CONTRACT NUMBER: AI20323 (NIAID)  
AI41325 (NIAID)  
DK52081 (NIDDK)  
DK58957 (NIDDK)  
SOURCE: Molecular microbiology, (2002 Jul) Vol. 45, No. 2, pp.  
277-88.  
Journal code: 8712028. ISSN: 0950-382X.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200209  
ENTRY DATE: Entered STN: 19 Jul 2002  
Last Updated on STN: 28 Sep 2002  
Entered Medline: 27 Sep 2002

AB Escherichia coli 0157:H7 causes diarrhoea, haemorrhagic colitis, and the  
haemolytic uraemic syndrome. We have identified a protein of previously  
unknown function encoded on the pO157 virulence plasmid of E. coli  
0157:H7, which is the first described protease that specifically cleaves  
C1 esterase inhibitor (C1-INH), a member of the serine protease inhibitor  
family. The protein, named StcE for secreted protease of C1 esterase  
inhibitor from EHEC (formerly Tagn), cleaves C1-INH to produce (unique)  
approximately 60-65 kDa fragments. StcE does not digest other serine  
protease inhibitors, extracellular matrix proteins or universal protease  
targets. We also observed that StcE causes the aggregation of cultured  
human T cells but not macrophage-like cells or B cells. Substitution of  
aspartic acid for glutamic acid at StcE position 435 within the consensus  
metalloprotease active site ablates its abilities to digest C1-INH and to  
aggregate T cells. StcE is secreted by the etp type II secretion pathway  
encoded on pO157, and extracellular StcE levels are positively regulated  
by the LEE-encoded regulator, Ler. StcE antigen and activity were  
detected in the faeces of a child with an E. coli 0157:H7 infection,  
demonstrating the expression of StcE during human disease.  
Cleavage of C1-INH by StcE could plausibly cause localized  
pro-inflammatory and coagulation responses resulting in tissue damage,  
intestinal oedema and thrombotic abnormalities.

L3 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:1019513 HCAPLUS  
DOCUMENT NUMBER: 141:421057  
TITLE: E. coli 0157:H7 C1-esterase inhibitor-binding protein  
StcE and its antibodies in diagnosing and treating  
enterohemorrhagic E. coli infection  
INVENTOR(S): Welch, Rodney A.; Lathem, Wyndham W.; Grys, Thomas E.  
PATENT ASSIGNEE(S): Wisconsin Alumni Research Foundation, USA  
SOURCE: U.S. Pat. Appl. Publ., 59 pp., Cont.-in-part of U.S.  
Ser. No. 2,309.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 3  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2004234530	A1	20041125	US 2004-786445	20040225
US 2002160433	A1	20021031	US 2001-2309	20011026
US 6872559	B2	20050329		
WO 2005083088	A1	20050909	WO 2005-US5943	20050225

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW  
 RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2006153828 A1 20060713 US 2005-294087 20051205  
 PRIORITY APPLN. INFO.: US 2000-243675P P 20001026  
 US 2001-2309 A2 20011026  
 US 2004-786445 A 20040225  
 US 2004-633583P P 20041206  
 US 2005-651560P P 20050210

AB Disclosed is a p0157 plasmid-specified polypeptide encoded by gene StcE found in E. coli EDL933 and other E. coli that binds to and cleaves C1-esterase inhibitor (C1-INH), and antibodies specific for the polypeptide. StcE protein contains a zinc metalloprotease active site. StcE is able to cleave both purified and serum-assocd. C1 inhibitor and inhibit classical complement-mediated erythrocyte lysis by potentiating C1-INH-mediated inhibition of classical complement. Mutagenesis confirms that glutamic acid 435 is necessary for both binding and cleavage of C1 inhibitor. Also disclosed are methods employing the polypeptide for diagnosing enterohemorrhagic E. coli infection, identifying potential inhibitors of its activity, and reducing viscosity of material contg. glycosylated polypeptides.

L3 ANSWER 5 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2005-25757 BIOTECHDS

TITLE: Novel purified antibody that binds specifically to C1-esterase of StcE producing bacteria e.g. enterohemorrhagic Escherichia coli O157:H7 strain, useful for treating infection caused by enterohemorrhagic Escherichia coli O157:H7;

StcE protein-specific antibody and  
 C1-esterase vaccine for C1-esterase-inhibitor and  
 bacterium epithelium cell colonization reduction

AUTHOR: WELCH R A; LATHEM W W; GYRS T E

PATENT ASSIGNEE: WISCONSIN ALUMNI RES FOUND

PATENT INFO: WO 2005083088 9 Sep 2005

APPLICATION INFO: WO 2005-US5943 25 Feb 2005

PRIORITY INFO: US 2004-786445 25 Feb 2004; US 2004-786445 25 Feb 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-619195 [63]

AB DERWENT ABSTRACT:

NOVELTY - A purified antibody (I) that binds specifically to a polypeptide comprising amino acid residues at position 24-886 of a fully defined 886 amino acid (SEQ ID NO: 2) sequences given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a composition (II) comprising a purified polypeptide comprising at least 25 consecutive amino acid residues of SEQ ID No: 2 and an adjuvant; (2) reducing (M1) complement-mediated disruption of cells, involves contacting the cells with a purified polypeptide comprising amino acid residues 24-886 of SEQ ID No: 2 or a fully defined 886 amino acid (SEQ ID NO: 19) sequences given in the specification (StcE E435D), to reduce complement-mediated disruption relative to that of cells not contacted with the purified polypeptide; (3) reducing (M2) the viscosity of a material comprising a mucin or a glycosylated polypeptide, involves contacting the material with a viscosity reducing effective amount of StcE; (4) a composition (III) for enhancing

delivery of a target antigen to mucosal cells, comprising the target antigen and StcE; (5) detecting StcE activity, involves contacting a sample containing or suspected of containing StcE with C1-INH under suitable conditions to allow cleavage of C1-INH by StcE, if present, and detecting C1-INH cleavage; and (6) evaluating a test substance for the ability to inhibit StcE, involves contacting C1-INH with the test substance and StcE, detecting the extent of cleavage of C1-INH, and comparing the extent of cleavage with that of C1-INH contacted with StcE in the absence of the test substance.

BIOTECHNOLOGY - Preferred Composition: In (II), the polypeptide comprises amino acid residues 24-886 of SEQ ID No: 2. Preferred Method: (M1) further involves contacting the cells with exogenous C1-INH. In (M2), the material is saliva or sputum.

ACTIVITY - Antibacterial; Antidiarrheic; Hemostatic; Antiinflammatory; Gastrointestinal-Gen. No supporting data is given.

MECHANISM OF ACTION - C1-esterase inhibitor.

USE - (I) is useful for reducing colonization of epithelial cells by StcE producing bacteria, which involves contacting the epithelial cells with (I) or an inhibitor of StcE. (I) is useful for detecting StcE in a sample, which involves contacting (I) with the sample and detecting binding of the antibody. (II) is useful for eliciting an immune response in an animal, which involves inoculating the animal with (II), to elicit an immune response. (III) is useful for eliciting in an animal an immune response to a target antigen, by contacting (III) the mucosal cells of the animal (all claimed). (I) is useful for treating enterohemorrhagic Escherichia coli O157:H7 infection, that cause diarrheal disease, hemorrhagic colitis, and haemolytic uremic syndrome (HUS).

EXAMPLE - No relevant example is given.(115 pages)

=> d his

(FILE 'HOME' ENTERED AT 11:05:01 ON 07 AUG 2006)

FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, EMBASE' ENTERED AT 11:05:31 ON 07 AUG 2006

L1 11 S STCE PROTEIN  
L2 9 DUP REM L1 (2 DUPLICATES REMOVED)  
L3 5 S L2 AND (VISCOSITY OR CLEAVING OR CLEAVAGE)

=> log y

COST IN U.S. DOLLARS	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	12.36	12.57
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE	TOTAL
	ENTRY	SESSION
CA SUBSCRIBER PRICE	-0.75	-0.75

STN INTERNATIONAL LOGOFF AT 11:09:01 ON 07 AUG 2006